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Biofilm formation capacity of *Salmonella* serotypes at different temperature conditions[1]

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ABSTRACT.- Borges K.A., Furian T.Q., Souza S.N., Menezes R., Tondo E.C., Salle C.T.P., Moraes H.L.S. & Nascimento V.P. 2018. **Biofilm formation capacity of *Salmonella* serotypes at different temperature conditions.** *Pesquisa Veterinária Brasileira 38 (1):71-76*. Centro de Diagnóstico e Pesquisa em Patologia Aviária, Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 8824, Porto Alegre, RS 91540-000, Brazil. E-mail: karen.borges@ufrgs.br

Salmonella spp. are one of the most important agents of foodborne disease in several countries, including Brazil. Poultry-derived products are the most common food products, including meat and eggs, involved in outbreaks of human salmonellosis. *Salmonella* has the capacity to form biofilms on both biotic and abiotic surfaces. The biofilm formation process depends on an interaction among bacterial cells, the attachment surface and environmental conditions. These structures favor bacterial survival in hostile environments, such as slaughterhouses and food processing plants. Biofilms are also a major problem for public health because breakage of these structures can cause the release of pathogenic microorganisms and, consequently, product contamination. The aim of this study was to determine the biofilm production capacity of *Salmonella* serotypes at four different temperatures of incubation. *Salmonella* strains belonging to 11 different serotypes, isolated from poultry or from food involved in salmonellosis outbreaks, were selected for this study. Biofilm formation was investigated under different temperature conditions (37°, 28°, 12° and 3°C) using a microtiter plate assay. The tested temperatures are important for the *Salmonella* life cycle and to the poultry-products process. A total of 92.2% of the analyzed strains were able to produce biofilm on at least one of the tested temperatures. In the testing, 71.6% of the strains produced biofilm at 37°C, 63% at 28°C, 52.3% at 12°C and 39.5% at 3°C, regardless of the serotype. The results indicate that there is a strong influence of temperature on biofilm production, especially for some serotypes, such as *S*. Enteritidis, *S*. Hadar and *S*. Heidelberg. The production of these structures is partially associated with serotype. There were also significant differences within strains of the same serotype, indicating that biofilm production capacity may be strain-dependent.

INDEX TERMS: *Salmonella* serotype, biofilm, temperature, poultry process, bacterioses.

RESUMO.- [**Capacidade de produção de biofilme por cepas de diferentes sorovares de *Salmonella* em quatro temperaturas de incubação.**] *Salmonella* spp. são um dos mais importantes agentes causadores de doenças transmitidas por alimentos em vários países, inclusive no Brasil. Produtos avícolas e ovos são os principais alimentos envolvidos na transmissão dos sorovares de *Salmonella* que são responsáveis por surtos de salmonelose em humanos. *Salmonella* possui a capacidade de formar biofilmes em diversas superfícies. O processo de formação de biofilme depende da interação entre as células bacterianas, a superfície de adesão e as condições do ambiente onde a bactéria se encontra. Estas estruturas favorecem a sobrevivência bacteriana em ambientes hostis, como em matadouros-frigoríficos e em indústrias processadoras de alimentos. Biofilmes são um grande problema em saúde pública, pois a ruptura destas estruturas pode provocar a liberação de microrganismos patogênicos e, consequentemente, a contaminação

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dos produtos. O objetivo deste estudo foi avaliar a capacidade de produção de biofilme por diferentes sorovares de *Salmonella* submetidos a quatro temperaturas de incubação. Cepas de *Salmonella* de 11 sorovares foram selecionadas. A produção de biofilme foi avaliada através do método de incubação em microplacas de poliestireno incubadas a 37°, 28°, 12° e 3°C. Estas temperaturas são importantes durante o ciclo de vida de *Salmonella* e para o processamento de produtos avícolas. Do total de cepas avaliadas, 92,2% foram capazes de produzir biofilme em pelo menos uma das quatro temperaturas testadas. Neste estudo, 71,6% das cepas produziram biofilme a 37°C, 63% a 28°C, 52,3% a 12°C e 39,5% a 3°C, independentemente do sorovar. Os resultados indicam uma forte influência da temperatura na produção de biofilme, especialmente para os sorovares *S*. Enteritidis, *S*. Hadar e *S*. Heidelberg. A produção de biofilme está parcialmente associada com o sorovar da cepa. Também foi observado que existe variação quanto à produção destas estruturas dentro de um mesmo sorovar, indicando que possivelmente a produção de biofilme é cepa-dependente.

TERMOS DE INDEXAÇÃO: sorovares de *Salmonella*, biofilme, temperatura, cadeia avícola.

INTRODUCTION

According to the World Health Organization (WHO), despite the growing concern regarding emerging pathogens in recent years, *Salmonella* spp. remain one of the most common agents of foodborne diseases in several countries, including Brazil (Brasil 2015, WHO 2016). The Centers for Disease Control and Prevention (CDC) estimate that approximately 48 million people get sick and 20,000 need to be hospitalized. Each year, almost 3,000 persons die because of foodborne illness in the United States. Of this total, approximately 1 million cases are caused by *Salmonella* (CDC 2011a). Poultry-derived products are the most common food products, including meat and eggs, involved in outbreaks of human salmonellosis and account for up to 47 % of all infections (CDC 2011b).

During the *Salmonella* life cycle, host colonization is alternated with periods of survival outside of the host (Winfield & Groisman 2003). To survive in this environment, *Salmonella* spp. have developed several mechanisms, including surface adherence and biofilm formation (Steenackers et al. 2012). Biofilms are complex communities composed of cellular organisms of the same or different species that are attached to both each other and an abiotic or biotic surface (Costerton et al. 1995, Donlan & Costerton 2002). This multicellular complex is surrounded by an extracellular matrix mainly composed of exopolysaccharides (Donlan & Costerton 2002, Hall-Stoodley & Stoodley 2009). The major components of the extracellular matrix are curli and cellulose. Curli fimbriae are involved in surface adhesion, cell aggregation, persistence in the environment, biofilm production, and host cell adhesion and invasion (Barnhart & Chapman 2006). Cellulose production confers adhesion to abiotic surfaces and bacterial cell-cell interactions (Römling 2007). The advantages of biofilm production include greater resistance to physical forces and the host immune system and toxic molecules like antimicrobials and disinfectants, metabolic cooperation and more efficient DNA sharing (Stewart & Costerton 2001, Hall-Stoodley & Stoodley 2009, Hung & Henderson 2009, Steenackers et al. 2012, Satpathy et al. 2016).

The biofilm formation process depends on interactions among bacterial cells (microbial shape, structure, molecular composition, presence of flagella, pili, capsules or exopolymeric substances), the attachment surface (chemistry, topography, and physicochemistry) and the environmental conditions (pH, nutrient availability, temperature, host proteins, and fluid dynamics) (Van Houdt & Michiels 2010, Campoccia et al. 2013, Cappitelli et al. 2014, Whitehead & Verran 2015). Temperature is one of the most important factors influencing the capacity to form biofilm (Stepanović et al. 2003, De Oliveira et al. 2014, Cabarkapa et al. 2015, Piras et al. 2015).

Biofilms favor bacterial survival in hostile environments, such as slaughterhouses and food processing plants (Steenackers et al. 2012). Animal food production systems are favorable places for biofilm formation because they offer ideal conditions for the development of these structures (Chmielewski & Frank 2003). Biofilm formation in the food industry can lead to economic losses due to facility and equipment impairments and food spoilage (Gram et al. 2007, Hung & Henderson 2009). Biofilms are also a major problem in public health because breakage of these structures can cause the release of pathogenic microorganisms and, consequently, product contamination (Costerton et al. 1995, Hung & Henderson 2009). The National Institutes of Health of the United States estimate that over 80 % of human bacterial infections could be related to biofilm formation (Hung & Henderson 2009).

Salmonella spp. have demonstrated the capacity to form biofilms on several surfaces including different types of stainless steel (AISI 304 or 316), polyethylene, polystyrene, acrylic, glass, and metal inert gas (MIG) and tungsten inert gas (TIG) melts (Casarin et al. 2014, Nguyen et al. 2014, Silva et al. 2014, Cabarkapa et al. 2015, Tondo et al. 2015, Casarin et al. 2016). Vestby et al. (2009) suggest that biofilm formation capacity may be an important factor for the persistence of *Salmonella* on food contact surfaces in the factory environment.

The influence of *Salmonella* serotypes on biofilm production capacity has not yet been completely elucidated, and there are few studies that compare the differences among the serotypes. Characteristics inherent to a specific serotype can influence its capacity for biofilm production (Díez-García et al. 2012, Schonewille et al. 2012, Castelijn et al. 2013). However, due to the variability within strains of the same serotype, it might not be possible to demonstrate this correlation (Agarwal et al. 2011, Lianou & Koutsoumanis 2012).

In this context, the aim of this study was to evaluate and compare the biofilm-forming capacities of *Salmonella* serotypes at four different temperatures of incubation.

MATERIALS AND METHODS

Bacterial strains and inoculum preparation. A total of 243 strains of *Salmonella enterica* belonging to 11 different serotypes (Table 1) were used in this study. These strains were isolated between 1996 and 2013 from either poultry or food involved in salmonellosis outbreaks in southern Brazil. A complete antigenic characterization and serovar identification were performed by

Table 1. Biofilm production of *Salmonella enterica* strains according to serotype at 37°C, 28°C, 12°C and 3°C

Serotype	Total number of samples	Temperature (°C)	Production of Biofilm				Total number of producers (%)
			NP (%)	WP (%)	MP (%)	SP (%)	
S. Enteritidis	150	37°C	42 (28)	66 (44)	31 (20.7)	11 (7.3)	108 (72)
		28°C	51 (34)	68 (45.3)	30 (20)	1 (0.7)	99 (66)
		12°C	62 (41.3)	80 (53.3)	7 (4.7)	1 (0.7)	88 (58.7)
		3°C	97 (64.7)	51 (34)	2 (1.3)	0	53 (35.3)
S. Heidelberg	49	37°C	21 (42.9)	25 (51)	3 (6.1)	0	28 (57.1)
		28°C	23 (46.9)	21 (42.9)	5 (10.2)	0	26 (53.1)
		12°C	35 (71.4)	13 (26.5)	1 (2.1)	0	14 (28.6)
		3°C	32 (65.3)	16 (32.7)	1 (2)	0	17 (34.7)
S. Hadar	14	37°C	2 (14.3)	10 (71.5)	1 (7.1)	1 (7.1)	12 (85.7)
		28°C	8 (57.1)	5 (35.8)	1 (7.1)	0	6 (42.9)
		12°C	5 (35.8)	7 (50.1)	1 (7.1)	1 (7.1)	9 (64.3)
		3°C	4 (28.6)	9 (64.3)	0	1 (7.1)	10 (71.4)
S. Typhimurium	8	37°C	0	7 (87.5)	1 (12.5)	0	8 (100)
		28°C	2 (25)	6 (75)	0	0	6 (75)
		12°C	4 (50)	4 (50)	0	0	4 (50)
		3°C	3 (37.5)	5 (62.5)	0	0	5 (62.5)
S. Anatum	5	37°C	1 (20)	2 (40)	1 (20)	1 (20)	4 (80)
		28°C	1 (20)	2 (40)	2 (40)	0	4 (80)
		12°C	2 (40)	2 (40)	1 (20)	0	3 (60)
		3°C	2 (40)	3 (60)	0	0	3 (60)
S. Bredeney	5	37°C	1 (20)	3 (60)	1 (20)	0	4 (80)
		28°C	1 (20)	1 (20)	2 (40)	1 (20)	4 (80)
		12°C	2 (40)	0	1 (20)	2 (40)	3 (60)
		3°C	2 (40)	3 (60)	0	0	3 (60)
S. Agona	4	37°C	1 (25)	3 (75)	0	0	3 (75)
		28°C	2 (50)	2 (50)	0	0	2 (50)
		12°C	3 (75)	1 (25)	0	0	1 (25)
		3°C	4 (100)	0	0	0	0
S. Tennessee	3	37°C	1 (33.3)	2 (66.7)	0	0	2 (66.7)
		28°C	2 (66.7)	1 (33.3)	0	0	1 (33.3)
		12°C	3 (100)	0	0	0	0
		3°C	2 (66.7)	1 (33.3)	0	0	1 (33.3)
S. Infantis	2	37°C	0	2 (100)	0	0	2 (100)
		28°C	0	1 (50)	1 (50)	0	2 (100)
		12°C	0	1 (50)	1 (50)	0	2 (100)
		3°C	1 (50)	1 (50)	0	0	1 (50)
S. Brandenburg	2	37°C	0	0	2 (100)	0	2 (100)
		28°C	0	2 (100)	0	0	2 (100)
		12°C	0	2 (100)	0	0	2 (100)
		3°C	0	2 (100)	0	0	2 (100)
S. Schwarzengrund	1	37°C	0	1 (100)	0	0	1 (100)
		28°C	0	0	0	1 (100)	1 (100)
		12°C	0	0	1 (100)	0	1 (100)
		3°C	0	1 (100)	0	0	1 (100)

NP = no biofilm producer, WP = weak biofilm producer, MP = moderate biofilm producer, SP = strong biofilm producer.

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The bacterial isolates were kept frozen at -80°C in brain heart infusion broth (BHI) (Oxoid, Basingstoke, Hampshire, United Kingdom) and were supplemented with 15 % glycerin (Synth, Diadema, São Paulo, Brazil). The bacteria were retrieved from frozen culture stocks and were cultured overnight at 37°C in trypticase soya agar (TSA) (Merck, Darmstadt, Germany). All strains were subsequently transferred to trypticase soya broth without glucose (TSB) (Difco Laboratories, Detroit, Michigan, United States) and were incubated again at 37°C for 24 hours. To prepare the inoculum, McFarland standard No. 1 (Probac do Brasil, São Paulo, Brazil) was used as a reference to adjust the turbidity of the bacterial suspension in TSB without glucose to a concentration of 3×10^8 CFU/mL.

Quantification of biofilm formation. The biofilm formation capacities of *Salmonella* serotypes were investigated under different temperature conditions (37°, 28°, 12° and 3°C) using a micro-titer plate method. These temperatures were chosen due to their importance in the poultry production process: 37°C is the optimum temperature for *Salmonella* growth (Gast 2008), 28 °C is the optimum temperature for the expression of extracellular matrix components in some *Salmonella* serotypes (Barnhart & Chapman 2006, Römling 2007, Steenackers et al. 2012), 12°C is required by the Brazilian sanitary service in cutting rooms of broiler processing plants (Brasil 1998), and 3°C is the ideal average temperature of domestic refrigerators (Silva et al. 2008).

To evaluate biofilm formation, 200 µL of each bacterial suspension were inoculated in three wells of four sterile 96-well flat-bot-

tomed polystyrene plates. Negative control wells containing only TSB without glucose were inoculated in triplicate. For biofilm evaluation, we used biological triplicates. The plates were covered and incubated aerobically for 24 h at 37°C, 28°C, 12°C or 3°C. After incubation, the contents of the microtiter plate were poured off, and the wells were washed three times with 250 µL of sterile saline solution (0.9 %). The attached bacteria were then fixed with 200 µL of methanol (Nuclear, Diadema, São Paulo, Brazil) per well for 15 minutes, after which the plates were emptied and dried at room temperature. Then, the plates were stained with 200µL per well of 2% Hucker crystal violet for 5 min. The stain was removed, and the plate was washed under running tap water. The plates were air-dried. The biofilm was resuspended in 200µL per well of 33% glacial acetic acid (Nuclear). *Salmonella* Enteritidis ATCC 13076 was used as a reference strain for all temperatures of incubation. In addition, for each temperature, we included a non-producer and a producer strain. These strains belonged to the serotype *S.* Enteritidis and were selected from our stock collection. The optical density (OD) of each well was measured at 550 nm in an ELx800 Absorbance Reader (Biotek, Winooski, Vermont, United States). The optical density of each strain (OD_S) was obtained from the arithmetic mean value of the respective three wells. The strains were classified as previously described (Stepanović et al. 2004). The cut-off OD (OD_C) for the microtiter-plate test was defined as three standard deviations above the mean OD of the negative control. The strains were classified into four categories: no biofilm producer ($OD_S \leq OD_C$), weak biofilm producer ($OD_C < OD_S \leq 2\times OD_C$), moderate biofilm producer ($2\times OD_C < OD_S \leq 4\times OD_C$) or strong biofilm producer ($4\times OD_C < OD_S$).

Statistics. The data obtained were subjected to statistical analysis using PASW Statistic software (IBM, Hong Kong). Descriptive statistical analysis was used to determine the grouping of the samples according the biofilm formation capacity. The non-parametric chi-square (χ^2) was performed to analyze the distribution of the strains according to the biofilm capacity production and to compare the evaluated temperatures. One-Way ANOVA and the post hoc LSD test were carried out to compare serotypes. The significance was defined as $p < 0.05$.

RESULTS

Among the 243 analyzed strains, 224 (92.2 %) were able to produce biofilm in a minimum of one of the tested temperature conditions, but to various extents. A total of 174 (71.6 %) strains produced biofilm at 37°C, 153 (63%) at 28°C, 127 (52.3%) at 12°C and 96 (39.5%) at 3°C, regardless of the serotype. Among the producer strains, most of them were classified as weak, and just a small number were strong biofilm producers. Strong and moderate producer strains were more common when the incubations were performed at 37°C and 28°C, respectively. Biofilm production was independent of the sources of the analyzed samples (data not shown).

The results per serotype according to the incubation temperature are shown in Table 1. All serotypes produced biofilm at 37°C and 28°C, and all produced these structures at 12°C and 3°C, except *Salmonella* Tennessee and *S.* Agona, respectively. No strains of *S*. Heidelberg, *S.* Typhimurium, *S.* Agona, *S.* Infantis, *S.* Brandenburg and *S.* Tennessee were classified as strong producers, regardless of the incubation temperature.

There were no significant differences ($p>0.05$) in biofilm production for *S.* Typhimurium, *S.* Agona, *S.* Anatum, *S.* Bredeney, *S.* Tennessee, *S.* Infantis and *S.* Schwarzengrund at the four evaluated temperatures. For *S*. Enteritidis and *S*. Hadar, biofilm production varied significantly ($p<0.05$) among all temperatures. For *S*. Heidelberg, the differences in biofilm production were significant ($p<0.05$) at 37°C and 28°C compared with biofilm production at 3°C.

To compare biofilm production among serotypes, only those with five or more strains were included in the statistical analysis. There were significant differences ($p<0.05$) among the serotypes at all tested temperatures. At 37°C, *S.* Enteritidis differed from *S.* Heidelberg, whereas *S.* Heidelberg differed from *S.* Enteritidis and *S.* Anatum. At 28°C, *S.* Enteritidis differed from *S.* Heidelberg and *S.* Bredeney, whereas *S.* Heidelberg differed from *S.* Enteritidis and *S.* Bredeney, and *S*. Hadar and *S.* Typhimurium differed from *S.* Bredeney. At 12°C, *S.* Enteritidis differed from *S.* Heidelberg and *S.* Bredeney, whereas *S.* Hadar differed from *S.* Heidelberg and *S.* Bredeney, and *S.* Typhimurium and *S.* Anatum differed from *S.* Bredeney. At 3°C, *S.* Enteritidis and *S.* Heidelberg differed from *S.* Hadar.

The results for *S.* Enteritidis and *S.* Heidelberg at 37°C were selected to compare biofilm production within strains of the same serotype at the same temperature. In both cases, there were significant differences ($p<0.05$) within the two serotypes.

DISCUSSION

Laboratory conditions are favorable for *Salmonella* growth and may not be encountered in food processing facilities (Wang et al. 2013). However, these conditions represent a reproducible method to evaluate the biofilm formation capacity among *Salmonella* (Stepanović et al. 2000, Stepanović et al. 2004, Naves et al. 2008, Agarwal et al. 2011, Díez-García et al. 2012, Lianou & Koutsoumanis 2012). The applied technique permitted the detection of biofilm production by *Salmonella* strains. Incubation in TSB without glucose, a low nutrient availability broth, favored biofilm formation (Rodrigues et al. 2009, Agarwal et al. 2011).

The microtiter plates used for this *in vitro* experiment mimic some of the plastics used in poultry production and kitchens (Díez-García et al. 2012, Piras et al. 2015). Our findings indicate great capability in *Salmonella* biofilm formation, possibly related to the increased capacity of these strains to adhere to hydrophobic surfaces, such as polystyrene, compared to hydrophilic ones, such as glass and stainless steel, as previously demonstrated by other authors (Rodrigues et al. 2009, Tondo et al. 2010, Steenackers et al. 2012). These results are of particular concern because plastic materials are widely used on breeding farms, in poultry slaughterhouses, in the food processing industry and in kitchens (Díez-García et al. 2012).

The incubation temperatures evaluated in this study are important for the *Salmonella* life cycle and to the poultry-products process (Brasil 1998, Barnhart & Chapman 2006, Römling 2007, Gast 2008, Silva et al. 2008, Steenackers et al. 2012). A decrease in the incubation temperature can reduce bacterial growth but can also favor biofilm production because the strains are able to express components that were not produced under other conditions (Stepanović et al. 2003, Agarwal et al. 2011, Lianou & Koutsoumanis 2012,

Schonewille et al. 2012). For example, curli and cellulose production occurs only at temperatures below 30°C, especially between 25°C and 28°C (Gerstel & Römling 2003, Stepanović et al. 2003, De Oliveira et al. 2014, Cabarkapa et al. 2015, Piras et al. 2015). Biofilm phenotypes are correlated with the production of extracellular matrix components (Cabarkapa et al. 2015). This characteristic could explain the fact that some strains did not produce biofilm at 37°C but acquired this capacity at 28°C. Stepanović et al. (2003) observed increased biofilm production at 30°C compared to incubation at 37°C. Piras et al. (2015) reported a higher biofilm production at 22°C than at 35°C. These findings indicate that the factors involved in biofilm production have different responses, according to the temperature of incubation (Cabarkapa et al. 2015).

More than 50% of the *Salmonella* strains were able to produce biofilms at 12°C. This result should serve as a warning to the food processing industry, especially poultry slaughterhouses, because it is the temperature required by the Brazilian sanitary service in the cutting rooms of broiler processing plants (Brasil 1998). It is also important to emphasize *Salmonella*'s capacity to produce biofilms at 3°C, which is the average temperature of a kitchen's refrigerator (Silva et al. 2008); this finding is important because most of the foodborne diseases in Brazil occur in the consumer's residence and mainly due to maintenance of food at incorrect temperatures (Brasil 2015).

The influence of serotypes in biofilm production capacity by *Salmonella* strains has not yet been completely elucidated, and few studies have compared their differences. The specific characteristics of each serovar could increase or decrease their capacity to produce biofilm (Vestby et al. 2009, Díez-García et al. 2012, Schonewille et al. 2012, Castelijn et al. 2013, Wang et al. 2013). Vestby et al. (2009) suggested that the biofilm-forming capacity might be an important factor for the persistence of some *Salmonella* serotypes in the food processing industry. Serotypes *S.* Agona and *S.* Montevideo are supposed to be "good biofilm producers" and have been considered persistent in Norwegian feed-factories for a number of years. In contrast, *S.* Typhimurium is rarely isolated from factories, and the tested strains produced little biofilm. However, in our study, both *S.* Typhimurium and *S.* Agona seem to be mostly weak biofilm producers.

Only 14.4% of the *S.* Enteritidis strains were not able to produce biofilm at any of the tested temperatures. Other authors reported similar results, and this serotype has been described as the strongest biofilm producer (Schonewille et al. 2012, Puffal 2013). According to Cabarkapa et al. (2015), the frequent involvement of *S.* Enteritidis in salmonellosis outbreaks may be a consequence of the strong capacity of some strains to produce biofilms. It has been previously demonstrated that *S.* Enteritidis has higher adhesion capabilities on several surfaces that are usually used in the food industry, including different types of glass, stainless steel, polyethylene, polystyrene, polypropylene, granite, and MIG and TIG melts (Oliveira et al. 2006, Mafu et al. 2011, Casarin et al. 2014, Cabarkapa et al. 2015, Tondo et al. 2015, Casarin et al. 2016).

Most strains of *S.* Heidelberg, *S.* Hadar and *S.* Typhimurium were weak biofilm producers at all temperatures. These results are similar to previous findings (Marin et al. 2009, Vestby et al. 2009, Castelijn et al. 2013). It has been previously demonstrated that strains of *S.* Typhimurium produce more biofilms on stainless steel than on acrylic surfaces, which could explain the low adhesion to polystyrene (Nguyen et al. 2014). Our results indicate that *S.* Agona is a weak biofilm producer, which is different from what has been observed in previous studies (Vestby et al. 2009, Díez-García et al. 2012, Lianou & Koutsoumanis 2012, Corcoran et al. 2013). Few studies have evaluated the biofilm-forming capacity of *Salmonella* strains belonging to serotypes *S.* Tennessee, *S.* Brandenburg, *S.* Bredeney, *S.* Anatum, *S.* Infantis and S. Schwarzengrund. It was previously observed that most of these strains showed a low capacity to produce biofilms. These data were similar to those found by Agarwal et al. (2011) and Castelijn et al. (2013). However, these authors also studied a few examples of these serotypes, and the small differences observed could be due to the reduced sample size.

The significant differences ($p<0.05$) within serotypes indicates that even within a serovar, there are important variations in the capacity to produce biofilms, as previously described (Castelijn et al. 2013). It is possible that variability within a single serotype makes it difficult to compare the strains' biofilm production capacities (Agarwal et al. 2011, Lianou & Koutsoumanis 2012). According to Agarwal et al. (2011), the intrinsic characteristics of each strain, such as the presence of fimbriae, flagella, membrane proteins and other cellular appendages, could be more important than the environmental conditions for biofilm production.

CONCLUSIONS

Most *Salmonella* strains had the capacity to produce biofilms in microtiter plates at all tested temperatures.

The strains showed different behaviors at different incubation temperatures, demonstrating that there is a strong influence of temperature on biofilm production.

The production of these structures is partially associated with serotype.

The data obtained indicate that biofilm production is strain-dependent and that their intrinsic characteristics determine their potential to form these structures. However, the regulation of biofilm formation by *Salmonella* spp. strains are very complex and further studies are necessary.

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